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Enzyme amperometric sensor for the determination of cholinesterase inhibitors or activators

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Abstract

An enzyme amperometric sensor (EAS) based on immobilized cholinesterase (ChE) sensitive to ChE effectors (both specific and non-specific) is shown to be useful in enzyme immunoassay For example, a mink autoimmune (Aleutian) disease can be diagnosed with the EAS when an antigen is labelled with a ChE inhibitor When the ChE-containing membrane is modified by incorporation of the antigen to give an immunoenzyme EAS, this immunoassay can be performed on the basis of the steric shielding of the enzyme active sites with the immunocomplexes formed

Keywords Amperometry, Biosensors, Enzymatic methods, Immunoassay, Cholinesterase

The analytical application of the biologically important enzyme cholinesterase (ChE) offers broad possibilities for developing new means of analytical monitoring In previous papers [1,2] an enzyme amperometric sensor (EAS) was proposed for the determination of various ChE effectors (inhibitors or activators) [1,2] Two different approaches to the use of the analytical systems on the basis of ChE can be considered first, the use of the biosensor for the determination of ChE specific effectors, and second, the determination of non-specific effectors which can somehow affect the enzyme reaction (e g , by creating steric hindrances) Both approaches will be discussed in this paper

Most of the usual enzyme immunoassay tests are based on spectrophotometric or fluorimetric detection of an enzyme activity (an enzyme can play the role of an antigen or antibody label) [3,4] Breyer and Radcliff [5] first used an electrochemical technique to investigate the antigen–antibody reaction, the antigen being an azo-protein which was registered polarographically Heineman and co-workers [6,7] investigated the use of differential-pulse polarography for studying the binding of a hapten marked with an electroactive "tag" A number of reviews show the proliferation of the application of electroanalysis in immunoassays [8–12]

The effectors, or modulators, of enzymes can be used as a label for antigens or antibodies instead of enzymes [4] Enzyme modulator-mediated immunoassay (EMMIA) has been used for the determination of 2,4-dinitrophenyllysine [13], with horseradish peroxidase serving as the indicator enzyme The modulator was an antibody to horseradish peroxidase In another study [14], an antigen (thyroxine) was marked with an organophosphorus inhibitor (modulator) of acetylChE and the immunochemical reaction was monitored spectrophotometrically The use of the EAS in inhibitor EMMIA using the steric hindrance effect will be discussed

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